Trabecular Meshwork Glycosaminoglycans in Human and Cynomolgus Monkey Eyes

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The glycosaminoglycans (GAGs) extractable from the trabecular meshworks (TM) of human and nonhuman primate eyes have been analyzed by sequential enzymatic degradation and cellulose acetate electrophoresis. For comparison, similar extracts of the cornea, sclera, iris, and ciliary body have also been analyzed. The distribution of glycosaminoglycans in human and in cynomolgus monkey TMs are similar, although not identical. The human TM contains hyaluronic acid (HA), chondroitin-4-sulfate and/or 6-sulfate (CS), dermatan sulfate (DS), keratan sulfate (KS), heparan sulfate (HS), and an unidentified band of Alcian Blue staining material, which is resistant to the enzymes that we used. Based upon quantitation of the Alcian Blue staining intensities of extracted GAGs, which have been corrected by a relative dye-binding factor, the GAGs of the human TM include: 29.0% HA, 14.1% CS, 21.5% DS, 20.3% KS, and 15.0% HS. The cynomolgus monkey trabecular GAGs include: 12.8% HA, 14.3% CS, 15.2% DS, 42.1% KS, and 15.6% HS. Invest Ophthalmol Vis Sci 26:1320-1329, 1985

The preponderance of evidence suggests that the primary site of aqueous outflow resistance resides within the trabecular meshwork and possibly within the deep portion of the corneoscleral meshwork and/or the amorphous juxtacanalicular basement membrane near the canal of Schlemm.1-3 Extracellular matrix appears to comprise a significant portion of this tissue and probably of the aqueous outflow barrier in primates.5-13 Quantitative aqueous perfusion5-6 and histochemical7-13 studies with degradative enzymes have lead to the suggestion that glycosaminoglycans (GAGs) may be a key physiologic component of the resistance to the outflow of aqueous humor. The GAGs may reduce the functional diameter of the flow channels through the deep corneoscleral intertrabecular spaces and/or regulate flow through the juxtacanalicular basement membrane.

Recently, significant biochemical advances in the techniques for characterizing GAGs, and the proteoglycans of which they are usually a component, have been reported.14 Micro techniques have also been developed, which facilitate the analysis of GAGs from small tissues.15-20 These advances make the analysis of the unlabeled GAGs from the primate trabecular meshwork feasible.4-20

We have examined the extracellular matrix GAGs of the human and nonhuman primate trabecular meshwork as the initial step in studies intended to clarify the role that GAGs play in the regulation of aqueous outflow. In essentially all of the tissues studied to date, the GAGs are found as components of larger proteoglycans and proteoglycan aggregates; the GAGs appear to function only as a part of these larger molecules.14 The GAG distribution profile of the trabecular meshwork of enucleated primate eyes was determined as a prelude to studying these more complex proteoglycans and to serve as reference points for studies of the regulation of the biosynthetic and degradative activities of the human trabecular meshwork in explant organ culture.

Materials and Methods

Materials

Human eye bank eyes were obtained from 6 to 72 hr after enucleation and used immediately or stored frozen at −20°C. Cynomolgus monkey (Macaca fascicularis) and a few Rhesus monkey (Macaca mulatta) eyes were enucleated at autopsy and used immediately or stored frozen at −20°C. Hyaluronidase (Hyaluronate lyase, EC 4.2.2.1, from Streptomyces hyalurolyticus), Chondroitinase AC (Chondroitin sulfate AC lyase, EC 4.2.2.5, from Arthrobacter aurescens), Chondroitinase ABC (Chondroitin sulfate ABC lyase, EC 4.2.2.4, from Proteus vulgaris), Keratanase (Keratan sulfate endo-B-galactopyranosylglycanohydrolase, from Pseudomonas species), Heparitinase (heparitin sulfate lyase, EC 4.2.2.8, from Flavobacterium heparinum), keratan sulfate, chondroitin-4-sulfate, chon-
Methods

Extraction of GAGs from tissues: The trabecular meshwork, cornea, iris, ciliary body and a 5-mm ring of the sclera were removed from enucleated eye bank or cynomolgus monkey eyes and separated under a Wild M5D (Heerbrugg, Switzerland) dissecting scope. The tissues were blotted and weighed. Lipids were extracted for 24 hr with one change, 10 volumes each, of chloroform:methanol (2:1). The tissues were rinsed in acetone, dried, weighed, and digested with papain for 24 to 48 hr at 65°C. The papain incubation buffer was 5 mM ethylenediaminetetraacetic acid (EDTA), 2 mM cysteine hydrochloride, 200 mM sodium acetate at pH 5.5, and contained 50 units of papain per milliliter and 1 ml of incubation mixture per 10 eyes. The papain and other protein remaining were precipitated by incubation with 10% (final volume) trichloroacetic acid (TCA) for 2 hr at 4°C and were centrifuged for 15 min at 4°C and 12,000×g; the pellet was rinsed once and the pooled supernatants were applied to Bio-Gel P-6DG (desalting-gel) columns to separate TCA and other small compounds from the GAGs. The high molecular weight fractions were pooled, the GAGs were precipitated overnight at −20°C with three volumes of absolute ethanol containing 5% potassium acetate and centrifuged for 90 min at 4°C and 12,000×g. This partially purified fraction was then redissolved in water for cellulose acetate electrophoresis (CAE) or sequential enzymatic digestion.

Cellulose acetate electrophoresis: The various GAGs and standards were applied to cellulose acetate strips (Micronose Plus, Beckman Instruments, Fullerton, CA) with a 0.25-μl applicator and electrophoresed in a Beckman Microzone model R-101 electrophoresis cell at room temperature for 2 hr at 4 mA using 0.3 M cadmium acetate buffer (pH 4.1).16,18 Strips were stained for 15 min in 1% Alcian Blue, 5 mM sodium acetate in 50% ethanol; destained in 5% acetic acid in 10% ethanol16; and scanned without clearing on an Helena Laboratories Rapid-Scan (Beaumont, TX) or a Beckman model CDS-200 densitometer. Areas under the curves were determined on a Zeiss Digiplan MOP-3 image analyzer (Carl Zeiss, Inc., Oberkochen, West Germany). Uniform quantities of standards, usually hyaluronic acid and chondroitin-4-sulfate, were included on all runs for quantitative comparisons between runs. In separate experiments, a "relative dye binding factor" for Alcian Blue staining and interaction was determined for each type of commercially available GAG applied to CAE strips. Several concentrations of each GAG were scanned densitometrically and the areas were used to evaluate linearity within the ranges of interest and to obtain estimates of the dye binding factors of each type of GAG relative to HA for comparison with unknowns.

Identification of GAGs by sequential enzymatic digestions: Measurement of the GAG profiles of the respective eye structures was accomplished by a modified version of methods previously described.15,17,19 Enough sample or standard to produce good visible bands when stained after CAE using 0.25-μl applications, was dissolved in 30 μl of water and a 5-μl aliquot was removed for electrophoresis on CAE. The remaining 25 μl were diluted to 100 μl final volume with the first enzyme buffer to give the final concentrations given below, the enzyme digestion was completed and the reaction was terminated by the addition of 3 volumes of cold 5 mM potassium acetate in ethanol. The GAGs were precipitated overnight at −20°C, centrifuged at 12,000×g for 90 min at 4°C, the supernatant was removed and the pellet was dried and resuspended in 25 μl of water. A 5-μl aliquot was removed for CAE and the remainder was diluted to 100 μl with the next enzyme solution; the process was repeated removing a 5-μl aliquot and reducing the sample volume until all the steps were completed. We eliminated the TCA precipitation step after each treatment16,17,19 because we found that our dermatan sulfate standards were incompletely precipitated from 10% TCA solutions by ethanol (data not shown).

The sequence of treatment buffers (final concentrations), enzymes, incubation conditions (including our modifications of reported methods), and components degraded were:

1. Streptomyces hyaluronidase (5 TR units) in 20 mM sodium acetate, 2 mM EDTA, 150 mM sodium chloride, pH 5.0, was incubated with the sample for 5 hr at 60°C to degrade hyaluronic acid.21,22

2. Chondroitinase AC (1 unit) in 20 mM Tris (tris (hydroxymethyl) aminomethane), 50 mM sodium acetate, 100 mM sodium chloride, pH 8.0, was incubated with the sample for 5 hr at 37°C to degrade chondroitin-4 and 6-sulfates.23,24
Fig. 1. Cellulose acetate electrophoretic profiles of glycosaminoglycans extractable from human trabecular meshwork compared with standards. Extracted GAGs from human trabecular meshwork (panel I) were electrophoresed with standards to establish the electrophoretic migration profile. Panel 2 shows the migration of keratan sulfate and hyaluronic acid. Panel 3 shows the migration of chondroitin sulfate and dermatan sulfate. Panel 4 shows the migration of heparin sulfate. The electrophoresis was run in the direction of the arrow, i.e., from negative to positive. These are densitometric traces of the CAE strips after Alcian Blue staining without clearing.

3. Chondroitinase ABC (1 unit) in the same buffer as in step 2 (above) was incubated with the sample for 5 hr at 37°C to degrade dermatan sulfate. 23,24

4. Keratanase (1 unit) in 50 mM Tris, 50 mM sodium acetate, pH 7.4, was incubated with the sample for 5 hr at 37°C to degrade keratan sulfate. 25,26

5. To the remaining 5 µl of sample was added 2.5 µl of 1N HCl and 2.5 µl of 20% n-butyl nitrite in ethanol; this was incubated for 5 hr at room temperature; neutralized with 2.5 µl of 1N NaOH; diluted to 100 µl final volume with water; and ethanol precipitated as above. This process is reported to degrade heparin and heparan sulfate. 27,28

6. In some cases, pools of material from step 5 and pools of the original starting material were subjected to degradation by heparitinase before electrophoresis. Heparitinase (10 units) in 1 mM calcium chloride, 0.1 M sodium acetate, pH 7.0, was incubated with the sample for 6 hrs at 37°C to degrade heparan sulfate. 29

Testicular hyaluronidase was used to check the chondroitinase AC degradation for inhibition by dermatan sulfate. None was observed under the conditions detailed above. In pilot experiments with standards, the amount of each enzyme used was shown to be in excess, thereby ensuring complete degradation of each GAG. The data presented in this article represent two or more separate degradation experiments for each GAG from each species and tissue and two or more CAE analyses of each fraction using a total of 150 human and 180 cynomolgus monkey eyes. The standard error of the mean of the individual analysis values were never in excess of 10% of the individual values. The nondegradable material from the sequential enzymatic degradation process was also incubated with deoxyribonuclease I, ribonuclease A, and alpha amylase with no detectable changes (data not shown).

Results

CAE Profiles of the GAGs Extracted from Human and Cynomolgus Monkey Trabecular Meshworks and Surrounding Tissues

Figure 1 shows the CAE migration pattern of GAGs extracted from human trabecular meshwork as compared to the migration of the various standards. Three peaks were observed, one coincident with hyaluronic acid, one intermediate, and one broader peak covering the range of the other standards. These standards are separable, but due to variations in the degree of sulfation or other characteristics in the various tissues, they could not be used for absolute identification of the respective GAGs.

Figure 2 shows a comparison of similar electrophoretic profiles of the GAGs extractable from human and cynomolgus monkey trabecular meshwork, sclera, cornea, ciliary body, and iris. Hyaluronic acid, peak I, is detectable in all the tissues studied except cornea; band II is present in relatively high amounts in all of the tissues except sclera, although it may not be the same material in each tissue. The remainder of the GAGs run as a broader peak (III) and comprise a conglomerate of several individual GAGs, which require
Identification of Trabecular Meshwork GAGs Via Sequential Enzymatic Degradation

The electrophoretic profiles of the trabecular GAGs in aliquots removed after each step in the sequential enzymatic degradation are shown in Figure 3. These profiles show a comparison of the human and cynomolgus monkey GAGs in the initial, untreated sample and in the samples after the degradation of HA, CS, DS, KS, and HS, respectively. The HS and remaining peak were both resistant to the n-butyl nitrite treatment, although the HS was degraded by heparitinase. Hatched regions represent the amount removed by each treatment; the stippled area, which appears as a new peak area not present in the previous scan, actually reflects a change in the migration rate of the remaining GAGs. The final remaining peak is resistant to all of the treatments.

The TM of both species contain HA, although the human has relatively more than the monkey. Both contain significant amounts of CS and of DS, although...
Identification of GAGs from Surrounding Eye Tissues by Sequential Enzymatic Degradation

The four adjacent tissues were also analyzed, both to provide a comparison of the tissue GAGs and to aid in the establishment of the uniqueness of the trabecular meshwork GAGs. Figure 4 shows the results of the enzymatic degradation method as applied to extracts of the human and cynomolgus monkey sclera. This tissue is relatively low (<10%) in its total content of hyaluronic acid, heparan sulfate, and undegraded material; although the cynomolgus monkey has a significant amount of heparan sulfate, as determined by n-butyl nitrite treatment, and considerable KS. The human has much more CS and the migration is considerably different from the CS of the monkey, suggesting different degrees of sulfation. Both have an intermediate quantity of DS.

Figure 5 shows the results of enzymatic analysis of the human and cynomolgus monkey cornea. Hyaluronic acid is not detectable on these strips, although it can be seen when they are overloaded. Both species have an intermediate amount of CS and less DS. Treatment with keratanase removes considerable KS in each species and causes a major increase in the electrophoretic mobility of the material remaining after treatment (stippled area). HS is not detected by n-butyl nitrite treatment and a large peak of undegraded material with increased mobility remains.

Figure 6 shows the same analysis of human and cynomolgus monkey ciliary body. HA is detectable in small amounts; slightly more CS is present, and in the monkey its migration is increased by the treatment (stippled area). DS is the predominant GAG in both species and KS is present in small amounts in the monkey and not detectable in the human. Treatment with n-butyl nitrite did not reveal any HS in either species and a large peak of undegraded material resists all treatments.

Figure 7 shows the same analysis of the GAGs of the human iris. Cynomolgus monkey iris was not available in sufficient amounts for complete rigorous analysis, but the basic profile was similar to that for the human. HA is present, as are significant amounts of CS and DS. No KS or HS degradation is detectable and an intermediate amount of material remains after all of the treatments.

Percentage of the GAGs from Human and Cynomolgus Monkey Eye Tissues

The hatched areas in Figures 3-7 were measured to obtain an estimate of the percentages of each type of GAG that was degraded by each of the enzymes. These data are presented in Table 1. The “Total” column is the sum of all the other columns and its deviation from 100 is an indicator of the cumulative error in the mea-
Table 1. Comparison of the percentages of each tissue GAG based upon direct quantitation of alcian blue staining*

<table>
<thead>
<tr>
<th>Type of GAG</th>
<th>HA</th>
<th>CS</th>
<th>DS</th>
<th>KS</th>
<th>H/HS</th>
<th>Other</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human TM</td>
<td>25.4</td>
<td>13.6</td>
<td>20.9</td>
<td>15.3</td>
<td>17.8</td>
<td>9.5</td>
<td>102.5</td>
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<tr>
<td>Monkey TM</td>
<td>9.9</td>
<td>12.2</td>
<td>13.1</td>
<td>28.1</td>
<td>16.4</td>
<td>19.8</td>
<td>99.5</td>
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<tr>
<td>Human sclera</td>
<td>5.6</td>
<td>58.6</td>
<td>19.6</td>
<td>14.9</td>
<td>ND</td>
<td>5.9</td>
<td>104.6</td>
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<tr>
<td>Monkey sclera</td>
<td>6.0</td>
<td>18.8</td>
<td>10.9</td>
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<td>10.4</td>
<td>5.4</td>
<td>97.9</td>
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<tr>
<td>Human cornea</td>
<td>ND</td>
<td>27.2</td>
<td>14.8</td>
<td>32.6</td>
<td>ND</td>
<td>29.3</td>
<td>103.9</td>
</tr>
<tr>
<td>Monkey cornea</td>
<td>ND</td>
<td>17.9</td>
<td>12.5</td>
<td>29.8</td>
<td>ND</td>
<td>49.9</td>
<td>110.1</td>
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<tr>
<td>Human CB</td>
<td>10.3</td>
<td>15.7</td>
<td>26.9</td>
<td>ND</td>
<td>ND</td>
<td>40.4</td>
<td>93.3</td>
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<tr>
<td>Monkey CB</td>
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<td>2.0</td>
<td>22.5</td>
<td>7.8</td>
<td>ND</td>
<td>54.1</td>
<td>93.6</td>
</tr>
<tr>
<td>Human iris</td>
<td>13.7</td>
<td>31.7</td>
<td>28.0</td>
<td>ND</td>
<td>ND</td>
<td>25.0</td>
<td>98.4</td>
</tr>
</tbody>
</table>

* Values are for the percentages of total alcian blue staining material removed by each enzyme treatment. The last column is the sum of the previous columns and its deviation from 100 reflects the relative cumulative error in these values.

measurements. The standard error in the mean of the individual values was never over 10% of the individual means. These values represent the percentages of Alcian Blue staining lost after each enzyme treatment.

Since the different GAGs may be expected to bind different amounts of dye per dimeric unit, based upon the number and type of charged groups present, an alternative basis for the evaluations was also used. Several dilutions of known amounts of commercially available GAGs of each type were applied to CAE strips, stained, and scanned; the areas of these profiles were used to evaluate linearity and to obtain a "dye-binding factor" for each type of GAG. These factors were then normalized relative to the factor for HA. These relative dye-binding factors and the "corrected" values for the percentage each type of GAG contributed to the total trabecular GAGs are shown in Table 2. The validity of each of these two estimation methods is evaluated in the Discussion section. They are in moderate agreement and the relative dye-binding factors do not vary greatly from 1.00.

Hyaluronic acid is present in relatively high amounts in the human TM, in somewhat lower amounts in the monkey TM, in both scleras, ciliary bodies, and the human iris. We could not detect any hyaluronic acid in the cornea at these levels of sample application. Chondroitin sulfate is present in intermediate amounts in all of the tissues studied, although there is less in the monkey ciliary body and considerably more in the human iris and sclera. The percentages of dermatan and keratan sulfate are also intermediate, although KS is low in the iris and ciliary body and higher in the sclera, cornea and monkey TM.

Heparan sulfate, as detected by chemical degradation, is in low to undetectable amounts in all the tissues except the monkey sclera. Heparitinase digestion of the trabecular GAGs of both species demonstrates that HS is actually present at much higher levels than was indicated by chemical degradation. The relatively high charge density of this GAG is also apparent from its relative dye-binding factor. The "other" column is the amount of material, which resisted all of the degradative treatments, and with the exception of the sclera and cornea ran at exactly the same relative migration rate. In the sclera, there was only a small peak remaining and in the cornea, the remaining material migrates faster than it does before the keratanase treatment. The area of this peak is included in the "Other" column although it may have been partially degraded.

Discussion

The trabecular meshworks of both human and non-human primates contain significant amounts of the five major GAGs found in the extracellular matrix of other tissues, as well as an unidentified component(s) that is resistant to all the degradative treatments. This "Other" component may include the nonGAG oligosaccharide chains from proteoglycans and glycoproteins and possibly even some other unidentified cellular or extracellular material which binds Alcian Blue; it does not appear to be degraded GAGs and we could only speculate upon its nature. No strong preponderance of any GAG was observed. The resistance of trabecular HS to n-butyl nitrite and its degradation by heparitinase has been observed by other investiga-

Table 2. Relative dye binding factors and comparison of the "corrected" percentages of total trabecular GAGs*

<table>
<thead>
<tr>
<th>Type of GAG</th>
<th>HA</th>
<th>CS</th>
<th>DS</th>
<th>KS</th>
<th>H/HS</th>
<th>HS</th>
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<tr>
<td>Factors</td>
<td>1.00</td>
<td>1.10</td>
<td>1.11</td>
<td>0.86</td>
<td>1.36</td>
<td></td>
</tr>
<tr>
<td>Human TM</td>
<td>29.0</td>
<td>14.1</td>
<td>21.5</td>
<td>20.3</td>
<td>15.0</td>
<td></td>
</tr>
<tr>
<td>Monkey TM</td>
<td>12.8</td>
<td>14.3</td>
<td>15.2</td>
<td>42.1</td>
<td>15.6</td>
<td></td>
</tr>
</tbody>
</table>

* Values are for the percentage of the total GAGs that are removed by each enzymatic degradation. The relative dye binding factors for each GAG were determined for commercially available GAGs of each type as described in the Methods Section. The factor for HA was arbitrarily set at 1.00 and the others were normalized relative to it. The "Other" peak was not included in these calculations.
Based upon the quantitation of Alcian Blue staining with or without "correction," the human TM contains relatively high percentage of HA, DS, and HS, although there are also significant percentages of CS, KS, and undegradable material. The cynomolgus monkey TM contains relatively less HA but more KS and undegradable material than the human TM. These results in good qualitative agreement with the reports of others. Incorporation of radiolabel into HA, CS, DS, KS, and HS by human trabecular meshwork in organ or cell culture has been observed by various investigators. The quantitative values vary with the culture conditions and the assay method. As a rule, the relative production of HA is higher in dividing cell culture than the level of total HA in eye bank meshworks. This disparity may indicate that HA synthesis occurs at a relatively higher rate in dividing cells in

![Fig. 4. Electrophoretic profiles after sequential enzymatic degradation of glycosaminoglycans from the sclera. Human and cynomolgus monkey scleral GAGs were subjected to sequential enzymatic digestions, and treated identically to those in Figure 3, except that n-butyl nitrite was used in the last step instead of heparitinase (see caption to Fig. 3).](image)

![Fig. 5. Electrophoretic profiles after sequential enzymatic degradation of glycosaminoglycans from the cornea. Human and cynomolgus monkey corneal GAGs were subjected to sequential enzymatic digestions and treated identically to those in Figure 4. The stippled areas again represents a change in the migrational position rather than new material.](image)
culture than its concentration "in situ" would indicate.\textsuperscript{3,38,41}

The relative distributions of the GAGs in the adjacent tissues of the anterior chamber are similar to findings of other investigators.\textsuperscript{20,30,42} The heparitinase digestion used on the TM as a final step degraded much of the "Other" peak, which was resistant to n-butyl nitrite degradation. We assume that some of this peak in the other tissues is also sensitive to this enzyme, but have not verified this experimentally. The relatively high amounts of some of the GAGs make it difficult to detect the presence of relatively small amounts of other GAGs without heavy overloading of the CAE strips, eg, corneal HA. Therefore, our inability to detect a GAG should not be construed as proof of its absence, but merely as evidence that it is not present in large amounts. We should point out, also that our tissue does include the trabecular wall of Schlemm's canal and this may contribute one or more of the GAGs.

We should caution that the quantitations of GAGs by the two methods that we have used (percentage of Alcian Blue staining material and "corrected" per-
percentages based upon "relative dye-binding factors" for commercially available GAGs have certain limitations. Clearly, the extent of Alcian Blue staining will vary for each type of GAG, therefore, the amount of Alcian Blue staining material is not simply convertible to some number of mg of GAG. It is also unlikely that the sulfate and carboxyl groups of each GAG bind this dye with identical affinities. The extent of sulfation also varies with the type of GAG and for any specific type of GAG with the tissue source and possibly also in response to unknown physiologic conditions. GAGs also exhibit considerable microheterogeneity. Therefore, the comparisons based upon the "relative dye-binding factors" of commercially available GAGs should also be viewed with some caution. Without having standards of the specific GAGs from the same tissue and identical physiologic conditions, neither method can be used to obtain exact molar or milligram values for the individual GAGs. The similarity between the results based upon the two quantitative estimation methods, the agreement with radiolabel incorporation studies and the agreement of the relative dye-binding factors with predictions from the structures of the GAGs from other tissues do add credibility to these values.

Since there is detectable Alcian Blue and ruthenium red staining in several regions of the aqueous outflow pathway, we can only speculate upon the distribution of the individual GAGs within the TM. It seems likely, however, that some of these GAGs, as components of proteoglycans, are intercalated between the collagen molecules within the trabecular beams; some may be components of the trabecular wall of the canal of Schlemm; others may be located on the surfaces of the trabecular cells extending out into the trabecular spaces and some may be within the juxtacanalicular basement membrane-like region.

GAGs may contribute to the outflow resistance by reducing the functional diameters of the intertrabecular channels in the deep corneoscleral meshwork. Poiseuille's law, which is thought to describe the outflow resistance through the meshwork, contains a term for the functional pore diameter raised to the fourth power. This is conceptually appealing and recent photomicrographs by Richardson are suggestive of this type of GAG distribution. The amorphous juxtacanalicular basement membrane may also contribute to the aqueous outflow resistance. If this is a true basement membrane, heparan sulfate may be found in this region. By analogy with the kidney, we might expect that the HS proteoglycan would serve as the primary flow barrier in the TM. Further experiments, however, will be necessary to test this hypothesis.

We can conclude then, that the extracellular matrix of the primate trabecular meshwork is composed, at least in part, of different but significant amounts of each of the five common GAGs; HA, CS, DS, KS, and HS. One or more of these may contribute directly to the trabecular barrier of aqueous humor outflow.

**Key words:** outflow pathway glycosaminoglycans, sequential enzymatic degradation, primate trabecular meshwork

**Acknowledgments**

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A brief account of this work has been presented previously (see Acott et al).

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